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Characterization of Bioactive Compounds in Biomass of *Hericium erinaceus* (Hericium-MRL)

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Table 1 presents the centesimal and chemical characterization of *Hericium erinaceus* mushroom biomass (MB). The biomass powder has a water content of less than 8% on a fresh weight basis (FW). Its water activity (a_w) is approximately 0.32, indicating the free water available for chemical reactions and microbiological growth, confirming that MB is a stable and safe dry product. *H. erinaceus* MB is under Food Safety Certification (ISO 22000:2018; Registration Number: PT- 2019/GSA.0263PT-BIO-05.620-0008110.2023.001; GB-ORG-04; Serial Number: 01850/24). The centesimal analysis revealed that *H. erinaceus* possesses 93.1% carbohydrates on a dry weight basis (DW), which includes monosaccharides, disaccharides, and polysaccharides. Glucans are the most abundant carbohydrates, comprising 79.9% of carbohydrates. Glucans are known for their various biological functions, including the potential to modulate gut microbiota. The analysis also indicated that *H. erinaceus* contains 4.0% DW of protein and 1.1% DW of soluble protein. In terms of fat and ash content, this species has approximately 1.8% DW and 1.1% DW, respectively. From a nutritional perspective, *H. erinaceus* MB has low energy values: 382 kcal/ 100 g DW.

Table 1. Chemical composition of *Hericium erinaceus* mushroom biomass (MB).

	Parameter	Content
Centesimal composition	Water content (% fresh weight-FW)	7.64±0.04
	Carbohydrates (% of dry weight-DW)$^{+}$	93.05±0.15
	Protein (% DW)	4.03±0.24
	Soluble protein (% DW)	1.13±0.05
	Ash (% DW)	1.06±0.03
	Fat (% DW)	1.82±0.04
	a_w	0.32±0.01
	Energy (kcal/ 100 g DW)	381.65±0.10
Carbohydrate profile	Total glucose (% DW)	75.76±3.93
	Total free sugars^{++} (% DW)	0.36±0.06
	Total glucans (% DW)	79.87±0.57
	(1→3)(1→6)-β-glucans (% DW)	2.89±0.55
	α-glucans and β-glucans with other linkages (% DW)	76.98±4.33

$^{+}$Carbohydrates were calculated by 100 minus the humidity, ash, total fat, and protein difference. $^{++}$Total sugars were calculated by the sum of quantified sugars. All determinations were carried out in triplicate.

Table 2 displays *H. erinaceus* MB's free (FAA) and total amino acids (TAA) profiles. The amino acid (AA) composition reveals a wide diversity, with the presence of several essential AAs. Gamma-aminobutyric acid (Gaba), an important neurotransmitter, was also quantified in this species (2.88 mg/ 100 g DW). The most prevalent AAs are glutamic acid (Glu), aspartic acid (Asp), arginine (Arg), alanine (Ala), and leucine (Leu). In addition to the key role of essential AAs, the identified AAs possess several health benefits, such as promoting muscle health and normal central nervous system function, as well as enhancing metabolism, energy homeostasis, immunity responses, and memory.

Table 2. Free (FAAs) and total amino acids (TAAs) composition of *Hericium erinaceus* mushroom biomass (MB).

AAs	FAA content (mg/ 100 g DW)	TAA content (mg/ 100 g DW)
Asp	N.D.	771.90±11.54
Glu	3.60±0.55	797.34±9.50
Gly	2.83±0.45	392.13±0.03
Thr*	4.20±0.53	405.55±4.48
Arg	N.D.	566.27±6.89
Ala	3.96±0.58	525.58±3.06
Tyr	0.64±0.02	342.98±1.92
Val*	1.38±0.20	414.47±5.39
Met*	N.D.	62.03±0.05
Phe*	1.47±0.20	298.96±2.15
Ile*	0.83±0.12	451.66±12.70
Leu*	4.14±0.00	511.60±3.61
Gaba	2.88±0.25	2.88±0.25
Total^{+}	28.25±1.75	5618.07±429.63

*Essential amino acids. $^{+}$Total AAs were calculated by the sum of quantified FAAs and TAAs. N.D. not determined. All determinations were carried out in triplicate.

Table 3 shows the mineral composition of *H. erinaceus* MB. The mineral profile reveals that this species is a valuable source of phosphorus (P), magnesium (Mg), and potassium (K). The European Food Safety Authority proposes that the adequate intake of P, Mg, and K for adults is 550, 300, and 3500 mg/ day, being the MB a good source of these minerals. *H. erinaceus* MB also possesses a low sodium-to-potassium (Na:K) ratio (approximately 0.03), which is associated with preserving cardiovascular health. These minerals play essential biological roles, including involvement in signaling, enzymatic, and metabolic processes, as well as immune, muscle, cardiac, and nervous system functions. Selenium (Se) was also quantified in considerable amounts (89.6 µg/ 100 g DW). This mineral is involved in numerous biological roles such as the immune system enhancement and metabolism of thyroid hormones.

Table 3. Mineral composition of *Hericium erinaceus* mushroom biomass (MB).

Mineral	Content (mg/ 100 g DW)
Mo	0.07±0.01
Zn	2.03±0.05
Cd	0.02±0.00
P	314.00±5.18
Ni	0.01±0.00
Mn	2.38±0.03
Fe	0.39±0.03
Mg	118.77±1.18
Ca	22.05±0.26
Cu	0.27±0.00
Na	5.01±0.02
K	164.52±1.63
Se	0.09±0.02
Total[+]	629.54±7.21

[+]Total minerals were calculated by the sum of quantified minerals. All determinations were carried out in triplicate.

Table 4 presents the fatty acid (FA) composition of *H. erinaceus* MB. This species possesses a rich FA profile, with key bioactive FA such as oleic and linoleic acids associated with normal brain and cardiovascular functions. The monosaturated FAs (MUFAs) and polyunsaturated FAs (PUFAs), both known for their health benefits, were found in higher concentrations than saturated fatty acids (SFAs), resulting in an unsaturated FAs (UFA): SFA ratio of 3.40.

Table 4. Fatty acid composition of *Hericium erinaceus* mushroom biomass (MB).

Fatty acid	Content (mg/ 100 g DW)
Myristic acid (C14:0)	7.63±0.72
Pentadecanoic acid (C15:0)	1.02±0.05
Palmitic acid (C16:0)	408.45±42.13
Palmitoleic acid (C16:1 c9)	2.32±0.12
Heptadecanoic acid (C17:0)	1.75±0.15
Stearic acid (C18:0)	50.60±5.54
Elaidic acid (C18:1 t9)	2.46±0.13
Oleic acid (C18:1 c9)	862.08±73.99
cis-vaccenic acid (C18:1 c11)	20.58±1.86
c18:1 c4/t6	2.40±0.17
Linoleic acid (LA) (C18:2 c9c12)	658.35±50.31
Arachidic acid (C20:0)	5.80±0.11
γ-linolenic acid (C18:3 c6c9c13)	16.77±0.88
Paullinic acid (C20:1 c13)	22.06±2.27
α-Linolenic Acid (ALA) (C18:3 c9c12c15)	17.34±1.41
C18:2 t9t11	0.77±0.02
Behenic acid (C22:0)	1.00±0.03
Dihomo-γ-linolenic acid (DGLA) (C20:3 c8c11c14)	0.84±0.03
C20:3 c11c14c17	14.2±1.53
α-eleostearic acid (C18:3 c9t11t13)	2.41±0.10
Eicopentaenoic Acid (EPA) (C20:5 c5c8c11c14c17)	6.16±0.05
Catalpic acid (C18:3 t9t11t13)	1.90±0.08b
Tricosanoic acid (C23)	3.06±0.13
C22:2 c13c16	3.48±0.16
Lignoceric acid (C24:0)	31.57±3.59
Docosahexaenoic acid (DHA) (C22:6 c4c7c10c13c16c19)	2.55±0.10
Total SFAs[+]	510.88±52.10
Total MUFAs[+]	891.01±76.27
Total PUFAs[+]	724.71±53.99
Total fatty acids (FAs)[+]	2148.66±184.50
Ratio unsaturated fatty acids: SFAs	3.40
Ratio MUFAs: SFAs	1.90
Ratio PUFAs: SFAs	1.50

[+]Total SFAs, MUFAs, PUFAs, and FAs were calculated by the sum of respective quantified FAs. All determinations were carried out in triplicate.

Table 5 displays the concentration of some bioactive macromolecules that play key roles in human health (e.g., antioxidant and anti-inflammatory), such as total free and bound phenolic compounds, carotenoids, ergosterol, and tocopherols (vitamin E isomers).

Table 5. Content of different bioactive groups of *Hericium erinaceus* mushroom biomass (MB).

Bioactive group	Compound	Content
Phenolic	Total free phenolics[1]	30.57±3.26
	Total bound phenolics[1]	25.40±2.95
Antioxidant capacity	Total free fraction[2]	1857.03±84.88
	Total bound fraction[2]	2253.84±364.13
Carotenoids	Total carotenoids[3]	9.27±0.84
Sterols	Ergosterol[4]	1.57±0.07
E vitamin isomers	α-Tocopherol[5]	410.81±45.80
	β-Tocopherol[5]	29.17±0.96
	γ-Tocopherol[5]	312.42±2.48
	δ-tocopherol[5]	64.35±2.48
	Total tocopherols[5,+]	816.74±42.17

[1] mg gallic acid equivalent/ 100 g DW; [2] mg ISH equivalent / 100 g DM; [3] mg β-carotene equivalent/ 100 g DW; [4]mg/ 100 g DW; [5]µg/ 100 g DW. [+]Total tocopherols were calculated by the sum of quantified tocopherols. All determinations were carried out in triplicate.

In addition to the rich content of glucans, this report highlights the significant presence of bioactive compounds in this MB species, especially Asp, Glu, P, oleic, and linoleic acids, as well as tocopherols. This bioactive profile underscores the potential health benefits of *H. erinaceus* MB, which contributes to vital human health, including brain, cardiovascular, and kidney functions.